Protocol for Western Blot for His-tagged recombinant protein

Prepare the transfer buffer: 25 mM Tris, 192 mM glycine, pH 8.3, 20% (v/v) methanol.

Note: Do not adjust the pH of transfer buffers by HCl or NaOH.

1. Immerse the PVDF membrane in 100% methanol for a few seconds.
2. Equilibrate gels and membranes in transfer buffer for 2-3 minutes.
3. Assemble the gel and membrane sandwich (Anode-Filter paper-Membrane-Gel-Filter paper-Cathode), roll out all the air bubbles.
4. Transfer for 30 min at 100mA each gel.
5. Block the unbound sites of the membrane with blocking reagents (5% (m/v) non-fat milk dissolved in PBST).
6. Incubated with the primary antibody (6x-His Tag Antibody) for 1h at room temperature and then 4℃ overnight.
7. Wash the membrane with PBST three times.
8. Incubated with the second antibody (Goat anti-Mouse antibody, HRP conjugate) for 1h at RT.
9. Wash the membrane with PBST three times.
10. Add the DAB Substrate Solution to the membrane and incubate until the desired development is achieved.